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Evaluation of Spermicidal Activity of Extracts from the Pericarp of *Sapindus saponaria* L. (Sapindaceae)

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Abstract

Objective: The analysis was performed to evaluate the spermicidal activity of extracts obtained from the pericarp of the fruit of Sapindus saponaria L. in goat sperm samples.

Methods: The fruits were dried, milled and macerated in ethanol:water 9:1 mixture, and concentrated in a rotary evaporator under reduced pressure to obtain the crude extract. One part of this extract was lyophilized, and another part was hydrolyzed in acidic medium. The presence of saponins in the extract was characterized by Fourier-transform infrared spectroscopy (FTIR). Spermicidal activity was evaluated in both extracts using goat semen sample and staining with eosin.

Results: The maximal spermicidal effect (89.4%) was observed with 5 mg/mL solution of the crude extract, while the 5 mg/mL solution of the hydrolyzed extract showed 77.62% activity. Only 32.6% activity was obtained with 0.5 mg/mL crude extract and 7.9% activity with 0.5 mg/mL hydrolyzed extract.

Conclusions: Saponins isolated from Sapindus saponaria L. may be useful spermicides of natural origin.

Keywords: spermicidal activity, saponins, infrared spectroscopy, active extracts.
Jel Code: N56.

1. Introduction

Saponins are secondary metabolites produced mostly by plants and some marine organisms where they have important biological roles in innate plants immunity. They production increases when pathogen infections stimulate chemical changes that convert their respective precursors into saponins (Faizal y Geelen, 2013). Most of the biological properties of saponins are due to their ability to cause structural changes and deformations, such as pore formation on the cell membrane; this inevitably causing cell lysis (Lorent et al., 2013).

Recent studies report saponins with multiple properties of biological activity such as: antivirals against the Herpes simplex virus type 1 (HSV1) and the Human Immunodeficiency virus (HIV), mediated by different mechanisms of action (De Groot y Müller-Goymann, 2016); anti proliferative by activating caspase-3, a very important protein in the apoptosis cascade, triterpene saponins from *Sapindus mukorossi* induce programmed cell death in human lung carcinoma cells (Zhang et al., 2015). In another study, Rashed et al. (2013) demonstrated the ability of a hydro-methanolic extract of *Sapindus saponaria* L. to act as a cytotoxic against human cell lines of different types of carcinomas: colon (HCT-15), cervical (HeLa), breast (MCF-7) and lung (NCI-H460). Other extracts of *Sapindus saponaria* L. have been tested against the larvae of the *Aedes aegypti* mosquito (Rojas et al., 2015), proving to be effective in different larval stages. It has also been demonstrated the capacity of saponins from the plant *Furcraea hexapetala* to inhibit the activity of the insect *Cylas formicarius*, which is the main pest affecting the sweet potato (*Ipomoea batatas*) crop (Rodríguez, 2016).

The antimicrobial activity of saponins has been reported in research such as those of Rashed et al. (2013), they showed that a hydro-methanolic extract of *Sapindus saponaria* L. has promising antimicrobial activities on bacteria such as *Bacillus cereus* and *Staphylococcus aureus*; antifungals as highlighted by Tsuzuki et al. (2007) in their study, reporting that the hydro-alcoholic extract of *Sapindus saponaria* L. had great activity against strains of *Candida albicans*. According to De Groot & Müller-Goymann (2016), the saponins of the onions (*Allium cepa* L.) alliospiroside A and alliospiroside B have the highest antifungal activity. There are also reported studies in fungi that infect fruits, such as papaya, which is usually attacked by the fungi *Colletotrichum gloeosporioides*, forming a type of anthracnose; treatments with extracts of the leaves of *Sapindus saponaria* L. proved to have potential to be used as natural antifungals (Marinho et al., 2018).

Many other studies have been published related to the capacity of other biological activities of saponins: molluscicides (Ribeiro et al., 1995; Huang et al., 2003), antiprotozoa (Guerra et al., 2008), anti-inflammatory (De Groot and Müller-Goymann, 2016; Yu et al., 2016), and hypoglycemic agents (Cartagena Ramírez, 2010; El Barky et al., 2017). Regarding spermicidal activity, Bhosale et al., (2013) reported the use of *Sapindus mukorossi* saponins in a spermicidal cream called CONSAP, which has already approved clinical phase III in India. Furthermore, animal studies did not show irritation in any part of the vagina or urinary tract, any saponins were detected in blood (Tiwari et al., 2008). Furthermore, Damke et al. (2013) conclude in their study that an ethanol-water extract of *Sapindus saponaria* can immobilize 100% of sperm at concentrations of 2.5 mg/mL, and they

demonstrated that at these concentrations the *in vitro* growth of *Lactobacillus acidophilus* is not affected.

Phytochemical studies of the genus *Sapindus* have identified more than 103 compounds, including flavonoids, triterpenoids, glycosides, carbohydrates, fatty acids, phenols, and saponins. Most of these saponins contain Hederagenin as their sapogenin. Although in a smaller measure, oleanolic acid is also reported as the aglycone of these saponins (Sachin et al., 2014). Murgu & Rodrigues-Filho (2006) detected 30 different saponins and 63 acyclic sesquiterpenes oligoglycosydes (ASOGs) in the fruits of *Sapindus saponaria* L. The objective of this investigation is to evaluate the spermicidal activity of the hydroalcoholic extract from the fruit pericarp of *Sapindus saponaria* L. with goat sperm.

2. Materials and Methods

2.1. Material Preparation and Extraction

The fruits from the pericarp of *Sapindus saponaria* L. were washed with water and the seeds were removed. Approximately 670 g of these fruits were dried in an oven at 40 °C for 4 days and then grounded in a mesh of 4 mm in a SM 100 RETSCH® mill. This material was oven dried at 40 °C for 48 hours; once dried, it was macerated with an ethanol-water mixture in a 9:1 ratio (Tsuzuki et al., 2007) for 4 days. This maceration process was repeated 3 times, the extractions were mixed and then concentrating under vacuum on a rotary evaporator at 45 °C and lyophilized. The final concentrate obtained was called crude extract. A part of this crude extract was treatment with acid hydrolysis; 150 mL of the extract was refluxed for 2 h with 750 mL of 5% sulfuric acid-ethanol solution. After reflux, the pH was adjusted between 6 and 7 with a solution of 5% potassium hydroxide in ethanol and 100 g of activated carbon was added to reflux it again for 30 min. The hydrolyzed extract was filtered under vacuum in Büchner funnel through a patch of 2 g of diatoms, the filtrate was concentrated under vacuum with a rotary evaporator at 45 °C, and finally lyophilized until obtaining a solid with a high presence of saponins (Ohgi et al., 1999; Rodríguez-Hernández et al., 2015).

2.2. Dissolutions Preparation

Solution of 10 % were prepared from crude and hydrolyzed extract. 5% and 0.5% solutions were obtained by diluting with distilled water. The pH of solutions from the hydrolyzed extract were adjusted around 7 with 2% sodium bicarbonate, to increases saponins solubility in water.

2.3. Infrared Spectra FTIR Analysis

Detections of functional groups characteristic of saponins was performed with infrared spectroscopy analysis to crude extract, scanning from 4000 to 700 cm^{-1}. Chloroform was used as solvent.

2.4. Spermicidal Activity

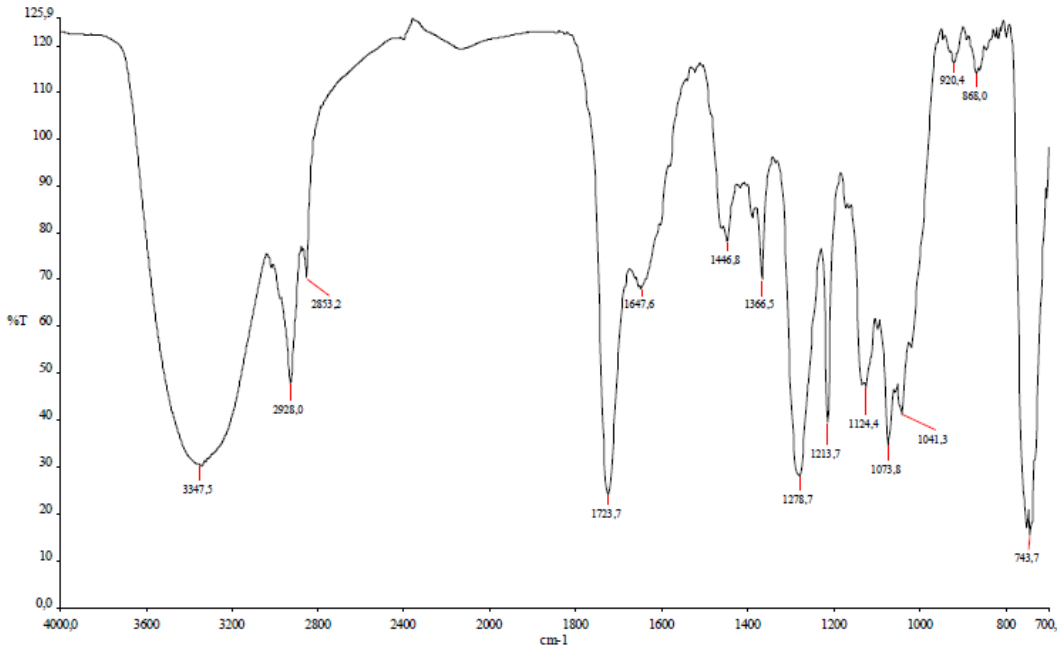
The goat sperm samples were donated by the Santa Lucía Experimental Farm, from the Department of Agricultural Science of Universidad Nacional (UNA), and the artificial vagina technique was applied. The samples were analyzed by macroscopic evaluation of pH, color, and volume in the Department of Chemistry of UNA. Microscopic evaluations were carried out with 300 µL of semen and then diluting them with 5700 µL of distilled water. A Neubauer chamber and 1% eosin staining were used for the sperm count. This first value was taken as a control test of the vitality percentage of the sample. The count was made in 12 quadrants for each trial: 10 µL of semen were used, 10 µL of the extract to be evaluated and 10 µL of eosin were taken. The spermicidal activity was immediately evaluated.

3. Results and Discussion

3.1. Infrared Analysis

The FTIR spectrum obtained from the crude extract can be seen in Figure 1. An intense and rounded band can be observed around 3347 cm^{-1} characteristic of hydroxyl -OH groups. A band near 2928 cm^{-1} is typical of carbon and hydrogen sp^3 bonds, the signal at 1723 cm^{-1} is characteristic of carbon oxygen sp^2 elongation from carbonyl group C=O. The signal of 1070 cm^{-1} is a sp^3 carbon-oxygen bond -CO and some signals around 1647 cm^{-1} corresponding to C=C carbon-carbon double bonds (Pavia et al., 2016). All these signs are characteristic of the saponins structure.

Figure 1: Infrared spectrum from crude extract of *Sapindus saponaria* L.



3.2. Sperm Evaluation

Macroscopic evaluation of the sample showed a slightly yellowish whitish color, with approximate 1 mL of volume and a pH of 7. Microscopically, the vitality of the spermatozoa was determined by studying stained spermatozoa with eosin, placing 20 µL of semen sample and 20 µL of eosin (Table 1).

Table 1: Live and dead sperm count in the sperm vitality control test

Quadrant	Unstained Spermatozoids	Stained Spermatozoids
I	103	10
II	115	10
III	93	8
IV	79	7
V	107	4
VI	111	5
VII	104	2
VIII	107	7
IX	114	6
X	117	5
XI	121	3
XII	116	8
Total	**1287**	**75**

Results in Table 1 show the sperm vitality of the sample is 94.5%, indicating that it can be considered fertile because the value is above 90%.

3.3. Spermicidal Activity

The results of the tests with the 5 and 0.5% dilutions for both extracts are shown in Table 2 and 3. It is observed in Figure 2 how the staining of eosin allows to evaluate the spermatozoids membrane integrity; they have serious damage to their cell membranes when staining their heads (Damke et al., 2013).

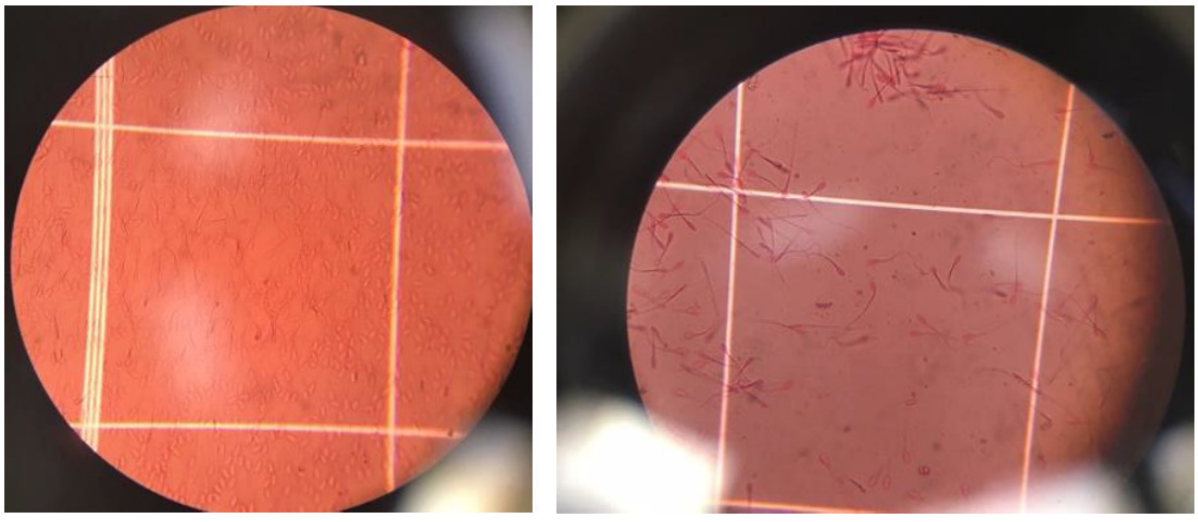
Table 2: Summary of results of spermicidal tests with the crude extract of *Sapindus saponaria* L

Concentration 0.5%		**Concentration 5%**	
Unstained spermatozoids	**Stained spermatozoids**	**Unstained spermatozoids**	**Stained spermatozoids**
287	139	33	278
Total: 426		**Total: 311**	
Vitality: 67.4% Spermicidal action: 32.6%		Vitality: 10.6% Spermicidal action: 89.4%	

Table 3: Summary of results of spermicidal tests with the hydrolyzed extract of *Sapindus saponaria* L

Concentration 0.5%		**Concentration 5%**	
Unstained spermatozoids	**Stained spermatozoids**	**Unstained spermatozoids**	**Stained spermatozoids**
679	58	64	222
Total: 737		**Total: 286**	
Vitality: 92.1% Spermicidal action: 7.9%		Vitality: 22.4% Spermicidal action: 77.6%	

Figure 2: Comparison of live sperm and dead spermatozoa. A) Live spermatozoids with white heads, not stained by eosin. B) Dead spermatozoids with red heads, stained by eosin



A) B)

Results in Tables 2 and 3 demonstrate the highest percentage of spermicidal activity is obtained in the most concentrated extracts, the effect being even greater with the crude extract (89.4%) than in the hydrolyzed extract (77.6%). Damke et al. (2013) reported a minimum effective concentration (MEC) of 2.5% for ethanol-water and butanol extracts, and 1.25% MEC for a purified sample of saponins from *Sapindus saponaria* collected in Brazil, which were tested with human sperm. In this

study the extracts demonstrated total immobilization of the sperm; 100% of sperm showed positive eosin staining and negative hyposmotic inflammation after treatment; this indicating complete membrane damage and death.

Sapindus mukorossi is a well-studied species used in medicine for its spermicidal activity (Gupta, 2005; Pelegrini et al., 2008), which has been associated with the presence of saponins in the plant (Garg et al., 1994; Maikhuri et al., 2003; Talwar et al., 2008). There are investigations of plants with spermicidal activity associate to surfactant structures present: we can mention Souad et al. (2007), they studied *Cestrum parqui* with positive spermicidal results *in vitro*; Dubey et al. (2011) reported immobilization of sperm in aqueous, methanolic and purified saponin extracts in *Ziziphus mauritiana*, besides Abu et al. (2011) concluded that the extracts of *Hymenocardia acida* had spermicidal properties. These investigations have demonstrated that plants with the presence of saponins are an interesting tool in the control of reproductive capacity. Future research is necessary to bring these natural structures to a level of patented pharmaceuticals and gain greater acceptance in the medical community.

Summary and Concluding Remarks

The extracts obtained present structures with functional groups consistent with the saponins. The infrared spectrum shows the presence of hydroxyls, carbonyls, and double bonds, which are associate to saponins structures. Crude and hydrolyzed extracts of *Sapindus saponaria* L. report important spermicidal activity at concentrations of 5%, the highest activity was found in the crude extract. Saponin purification processes by acid hydrolysis affects the spermicidal activity.

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Conflict of Interest Statement

We declare that we have no conflict of interest.

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